THE APPLICATION

OF

PHOTOGRAPHY TO MICROMETRY,

WITH

SPECIAL REFERENCE TO THE MICROMETRY OF BLOOD IN CRIMINAL CASES.

BY

J. J. WOODWARD, M.D., U.S.A.,

WASHINGTON, D.C.

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RECENT experiments in photographing the blood-corpuscles of man and other animals lead me to propose photography as affording the means of making comparative measurements more accurately, and with less expenditure of time, than can be done by any other method.

The plan I propose is simply as follows. The blood is placed on a glass stage micrometer and photographed with any convenient power, both blood and micrometer appearing sharply defined in the picture. The measurements are then made on the negative.

The stage-micrometer must be ruled on the upper surface of a piece of glass, and must have no thin cover. For mere comparative measurements any ordinary stage-micrometer in which the lines are equidistant can be made to answer by simply removing its cover; but if absolute values are aimed at, the micrometer must be compared with a recognized standard, and its constant error ascertained. The stage-micrometer used in the present series of experiments is the same described in a former paper.* It is ruled in hundredths, thousandths, and five-thousandths of an inch; and, so far as I have been able to ascertain by repeated comparisons of its parts with each other, the several divisions of each kind are equal throughout, five of the five-thousandths

* J. J. Woodward, On the similarity between the red blood-corpuscles of man and those of certain other mammals, especially the dog, etc. American Journal of the Medical Sciences, January, 1875, p. 151, and the Monthly Microscopical Journal, February, 1875, p. 65.

being equal to any one of the thousandths, and ten of the latter to any one of the hundredths.

On comparison with a standard scale belonging to the United States Coast Survey, by the contact method of Welcker, this micrometer proved, as I stated in the paper just cited, to have a constant error of +1.945 per cent.; that is, its lines are very nearly two per cent. too far apart; and this correction must of course be applied in the measurement of the photographs, as it was in the measurement of the corpuscles as seen in the microscope, which were published in the paper referred to.

The micrometer having been selected, it may be used for the measurement of blood dried in a thin film, of fresh blood in the moist state, or of dried stains soaked out by any selected method. In the first case the fresh blood should be spread on the micrometer by means of the edge of a glass slide, as proposed by Dr. Christopher Johnston, of Baltimore. A camel's-hair pencil is sometimes used for the same purpose; but it is a clumsy device, which no one who has been initiated into the proper method will ever employ. The blood thus spread may be photographed as seen uncovered, with a dry objective; but the best results are obtained with immersion objectives, to use which the specimen must of course be covered with a suitable piece of thin glass (.005″ to .008″ thick).

After two or three negatives have been made from the sample first selected, it is washed off, and a fresh specimen substituted, and so on. If it is desired to photograph fresh blood, a drop is put on the micrometer, a thin cover (of the thickness named) dropped on and allowed to press out the blood to the thinnest possible film. Excellent results may thus be obtained with the micrometer, almost as sharply defined as in the case of dried blood. If dried stains are to be examined, as in criminal cases, the fragments of dried blood are to be soaked out, by any of the approved methods, on the same micrometer, just as if on an ordinary glass slide; and when a satisfactory preparation has been made, it may be photographed, together with the micrometer, as in the former cases. For the purpose of soaking out such stains, I myself very much prefer to use a strong solution of caustic potash, as described by Virchow in 1857.* This reagent dissolves the fibrin and sets the red blood-corpuscles free without materially modifying them.

In order to obtain photographs of the specimens thus prepared, which shall at once be well defined and magnified sufficiently to render differential measurements easy, it is advisable to use immersion objectives of high power. I have employed the Powell and Lealand's one-sixteenth, and the one-eighteenth of Tolles, belonging to the Army Medical Mu-

* R. Virchow, Ueber die forensische Untersuchung von trockenen Blut-flecken. Virchow's Archiv, Bd. xii. S. 334.

seum, for this purpose, and, rejecting all eye-pieces and amplifiers, have aimed to obtain a magnification not less than one thousand diameters by distance alone. Of the two objectives named, the one by Tolles gives somewhat the sharper images, and has the flatter field. I have therefore used it for most of the work. It is only just to repeat here that, although correctly named a one-eighteenth, this objective is of lower power than the wet front of the Powell and Lealand one-sixteenth, belonging to the Museum, which is really a one-nineteenth, although the dry front of the same combination is a one-sixteenth. The chief difficulty in making photographs of blood-corpuscles with this high power is to avoid diffraction fringes in the images; but this can readily be done by following the method which I have explained in detail in my paper on "Photographing Histological Preparations by Sunlight."* This method is extremely simple, and offers no difficulties to any one who has acquired a reasonable degree of skill in microscopical manipulation. It is my desire to put it at the service of all sincere workers in this direction, and I cannot, therefore, view with indifference the publication of imperfect substitutes, which can only serve to waste the time of any one who may be misled into employing them. For this reason I feel it a duty to warn the reader against attempting to follow the methods described in two recent papers on this subject, published in the *Philadelphia Medical Times*.† It would be waste of time to criticise these papers in detail. The methods described in them appear to have been devised without any intelligent consideration of the optical principles involved. I was not therefore surprised, on seeing some of their author's photographs of blood-corpuscles with high powers, referred to in his second paper, to find them lacking in definition, full of diffraction fringes, and, by reason of these faults, unfit for measurement or any other serious use.‡ Nor was I surprised to read, near the end of the second paper, that "no one who has not tried it can form an idea of the difficulties attending the application of high powers to microphotography, and a single tolerably good negative is often the only result of a hard day's labor."

By my own easy method, on the other hand, an average morning's work produces from fifteen to eighteen successful negatives. The time

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* Surgeon-General's Office, July 13, 1871. Reprinted in the American Journal of Science and Arts, October, 1871, p. 258, the Monthly Microscopical Journal, October, 1871, p. 169, and the British Journal of Photography, October 27, 1871, p. 507.

† Carl Seiler, Photographic Enlargements of Microscopical Objects. Philadelphia Medical Times, June 5, 1875, p. 563. By the same: High Powers in Microphotography, op. cit., February 10, 1875, p. 249.

‡ Since writing the above, I have seen a photograph of blood by Dr. Seiler which is free from diffraction. It is, of course, taken with a much *lower* power than the others. Even this picture is *not sharp*, as it would have been had a proper method been used.

of exposure with the objectives named above, arranged to magnify the blood-corpuscles about 1000 diameters, is usually less than a second. A heliostat is therefore not *necessary* to obtain satisfactory results, as I have several times shown by taking such pictures quite as well without it as with it. The heliostat, however, is a great time-saver, since without it the light must be re-adjusted for every picture. I recommend any one about to experiment in this direction to procure one. An instrument which will answer every purpose can be extemporized for a few dollars out of a bit of looking-glass and the works of a Yankee clock; but even the cost of the admirable heliostat of Silbermann, as made by Dubosq, is only five hundred francs, which will be more than repaid by the time saved during the first year, unless the time of the experimenter is not worth as much as that of an ordinary day-laborer.

I also find it economical to employ a professional photographer in my dark-room, and I recommend others to do the same. By this plan the microscopist is left free to devote his sole attention to procuring the best possible optical images, while the photographer has nothing to consider but the best possible chemical work. A much greater quantity of good work can be produced in this way, in a given time, than is possible if the microscopist undertakes to do the photography himself. In this case he almost certainly sacrifices the optical part of the work to the photographic, or vice versa, and a reasonable degree of success is attained only by a wasteful expenditure of time.

Satisfactory photographs having been obtained, the corpuscles are next to be measured on the glass negatives. Paper prints are never exactly the size of the negatives. They spread a little if rolled; if not rolled, they may prove either a little larger or a little smaller than the negatives. Moreover, this spreading or contraction does not take place equally in all directions, and is sometimes quite irregular. It is most accurate, therefore, to measure on the negatives. For this purpose I lay the negative, with its varnished film uppermost, on the ground glass of an ordinary photographic re-touching frame, illuminated from behind by a mirror, and measure the corpuscles under a magnifying glass by means of a transparent scale ruled in hundredths of an inch on a thin strip of horn. The accuracy of this scale is of course determined by comparison with a standard. To avoid parallax, I turn the ruled side of the scale down so as to bring it in contact with the varnished film. It will very generally be found that the dried corpuscles are not perfectly round; the longest and shortest diameters must then be measured, and the mean taken. As the image of each corpuscle is measured, a dot of water-color is put on it, that it may not be measured a second time. This is readily washed off when the work is done. Extremely

deformed corpuscles, and those which are obviously turned on edge, are not measured; but no others are omitted. As each corpuscle is measured it is entered on a check-list, which shows, when all on the negative have been measured, the number of corpuscles of each size. The sum of all the values, divided by the number of corpuscles measured, gives the average size of the images of the corpuscles in one one-hundredths of an inch. This average size, divided by the true magnifying power, gives the true average size of the corpuscles. To find the true magnifying power, I measure the distance of the lines of the micrometer from centre to centre on the negative, and divide by their true distance apart on the micrometer (that is, their nominal distance apart corrected by the ascertained constant error of the micrometer). The number of corpuscles on each negative, in my experiments, has ranged, with a single exception, from 50 to 175. Had the power always been just 1000 diameters, the measurements in one one-hundredths of an inch would have corresponded to one one-thousandths of an inch, and could be relied upon as correct to that figure. That is, writing the results in decimals of an inch, any error of observation would have been less than one significant figure in the fifth decimal place. With slightly higher magnifications, the results are still more accurate. When it comes to computing the mean of from 50 to 175 such measurements, it can hardly be questioned that it is proper to carry out the mean results one decimal further, and it is not extravagant, therefore, to claim that the computed results are correct to the sixth significant figure. In the appended table, therefore, I give the average size of the corpuscles represented on each negative in millionths of an inch (together with the equivalent value in millionths of a millimetre, obtained by computation).

I feel justified in claiming for the method above detailed, that it requires less time for an equal number of measurements, and that it is more accurate than any of the methods heretofore employed for the micrometry of blood-corpuscles. I also claim for it that it is capable of useful application in the micrometry of many other objects for which great accuracy is desirable.

As to the time required, I suppose that twenty-five to fifty negatives, containing from 50 to 175 corpuscles each, can be made and the corpuscles measured in less than a quarter of the time necessary to measure the same number of corpuscles in the microscope by means of a glass eye-piece micrometer, and in less than a tenth of the time necessary if a cobweb micrometer be used.

As to accuracy, I have already mentioned the advantages of the plan proposed. I have next to refer to certain sources of inaccuracy in the method ordinarily employed. This method consists in the use of an

eye-piece micrometer to which values are first given by focusing on a stage-micrometer and comparing the two sets of lines; the stage-micrometer is then removed and replaced by the slide to be measured. But the values thus obtained are only true so long as all the optical conditions under which they were procured are rigorously maintained; and unintentional errors may be introduced in various ways. Especially must it be noted that the least alteration of the cover-correction of the objective, whether by accident or for the purpose of improving definition, will be found to modify the magnifying power of the objective, and, of course, to alter the value of the eye-piece micrometer. If, bearing this fact in mind, the observer first finds the best position of the screw-collar for the slide of blood to be measured, and then inserts the stage-micrometer, to give values to the eye-piece micrometer, he will very often discover, when he replaces the blood-slide and begins to measure it, that the cover is of different thicknesses in different parts, and that he must either change the correction or be content to measure the corpuscles as seen somewhat out of focus.

Besides this source of error, to which too little attention has been paid, there is another, which appears to have been altogether neglected. Most of the published measurements of blood-corpuscles have been made on blood dried in thin films on glass. But, however daintily this operation is performed, a large proportion of the corpuscles become more or less elliptical in drying. Yet, in the micrometry of the blood-corpuscles, as practised hitherto, they are measured in only one direction. Under these circumstances inaccurate results are of course inevitable.

With the comparatively low powers used by most of those who have published measurements of the blood-corpuscles, this source of error was naturally overlooked; but with a thousand diameters and upwards it becomes evident enough. In making the measurements reported in my former paper, I endeavored to escape this error by measuring only those corpuscles which appeared to be perfectly round. "Large and small forms were not searched for, but all the perfectly-formed corpuscles brought into view by the movement of the stage were measured as they passed under the micrometer, without selection, until the required number was recorded." I am now satisfied that the larger corpuscles are more frequently deformed than the smaller ones, and that, by pursuing the plan I did, I measured an undue proportion of the smaller corpuscles, and thus obtained averages somewhat less than the truth. In this way only can I account for the circumstance that the measurements now published are somewhat larger than those in my former paper, although the same micrometer was used, and with the utmost care.

Of course, since this source of error has been pointed out, it would

be possible to measure the longest and shortest diameter of the elongated corpuscles in the microscope as well as on the photographs; but it would require a much greater expenditure of time.

It is my purpose, when time permits, to prepare a series of photographs of moist blood, and of blood soaked out from dried stains, as in criminal cases. A few such photographs accompany the present paper, to show the perfect feasibility of these applications of my method. Especially is the application to criminal cases worthy of practice. Perhaps I ought not to go so far as to say that no expert who goes into court to testify about blood-stains deserves to be listened to by a jury unless he takes with him photographs of the blood examined on a stage-micrometer; but certainly it must be admitted that hereafter the most trustworthy expert-testimony in such cases will be that which is corroborated by photographic evidence. The general introduction of this severe method of recording accurately the facts observed, is the more desirable because of late a spirit of exaggeration seems to have possessed certain experts, who either boldly claim, or in ambiguous language obscurely insinuate, that they possess the power of discriminating human blood from that of other animals in the dried stains which are submitted to examination in criminal cases.

The latest offender in this direction is Malinin,* who published last year a paper in which, by the measurement of corpuscles soaked out from dried stains, he claims to distinguish not only between human blood and the blood of many other mammals, but, under certain conditions, between human blood and the blood of any other mammal, even the dog. To make out his case, Malinin assumes the invariable accuracy of Carl Schmidt's† mean values of the diameter of the blood-corpuscles of man and certain mammals, which he republishes without credit, as if they were his own. It is precisely this assumption of invariability which leads him astray. The truth is that not only do the individual corpuscles in every drop of blood vary considerably in size, but, as might be anticipated from this very fact, the average size obtained by measuring a limited number of corpuscles (50 to 175, still more in the case of but 10 to 50, as usually practised) varies considerably, not only between different individuals, but also between different parts of the very same drop of blood.

Now, it is true, as was shown by the measurements of human and dog's blood, published in my former paper, that the mean diameter of

* Malinin, Ueber die Erkennung des menschlichen und thierischen Blutes in trockenen Flecken in gerichtlich-medicinischer Beziehung. Virchow's Archiv, Bd. lxv. (1875) S. 528.

† Carl Schmidt, Die Diagnostik verdächtiger Flecke in Criminalfällen. Mittau und Leipzig, 1848. When I published my former paper, as I then stated in a foot-note, I had not been able to see a copy of this paper; but one has since been received at the Library of the Surgeon-General's Office.

the corpuscles in a given sample of human blood is often rather larger than the mean of a sample of dog's blood selected for comparison. I may even go further, and say that the average of all the measurements of human blood I have made is rather larger than the average of all the measurements of dog's blood. But it is also true that it is not rare to find specimens of dog's blood in which the corpuscles range so large that their average size is larger than that of many samples of human blood. This was clearly shown by the measurements published in my former paper, as it is by those which are appended to the present paper, and will be by any fair series of comparative measurements.

Thus I note here with pleasure that, since the publication of my former paper, Professor Gulliver, whose measurements of blood-corpuscles are those most frequently cited in English works, has published an extended and revised table of measurements of the blood-corpuscles of vertebrates, prefaced by some remarks, in the course of which he expressly affirms the futility of attempting to distinguish human blood in criminal cases.*

I conclude this paper with a table of measurements of the red corpuscles of man, the dog, and the guinea-pig, as dried in thin films on the glass micrometer, and measured by the photographic method above described. A print of each negative accompanies this paper, and copies of a selection of them will shortly be sent to convenient places in the larger cities, with the view of making them accessible to those who may be interested in this question.

The table gives the number of corpuscles measured on each negative, the diameter of the maximum and minimum, and the mean. The maxima and minima are given, like the mean, in millionths of an inch, because, although the measurements were actually made in hundredths of an inch, which with about one thousand diameters would correspond to about hundred-thousandths, yet the measures when corrected by the true magnifying power seldom remained a whole number, and the fraction is expressed by an additional decimal.

The measurements of human blood are from twenty-two negatives,

* George Gulliver, Observations on the Sizes and Shapes of the Red Corpuscles of the Blood of Vertebrates, etc. Proc. of the Zoological Society of London, June 15, 1875, p. 474. The passage referred to in the text is on page 484 : "As before noticed, the magnitude of the corpuscles in a single species, not excepting the human, is liable to variations within certain limits; and there commonly appear in one field of vision of the same corpuscles differences amounting to at least one-third larger and smaller than the average. Hence, as regards the medico-legal question, however truly a careful observer (Dr. Joseph G. Richardson, Monthly Micros. Journ., Sept. 1874) may have distinguished, by comparative measurements of the corpuscles, stains of human blood from those of the sheep or ox, this kind of diagnosis, as Dr. J. J. Woodward observes (Monthly Micros. Journ., Feb. 1875), would be ineffectual in some probable and more possible cases. It should be borne in mind, too, that in the apyrenæmata" (*i.e.*, the mammalia) "the membranous bases of the blood-disks, when deprived of their color by maceration in water, are about a third smaller than the unaltered corpuscles."

taken from nine drops of blood obtained from eight individuals, the whole number of corpuscles measured being 1766. The maximum size measured was 396 millionths of an inch in diameter. But two corpuscles of this great size were measured. The smallest corpuscle measured was 216 millionths of an inch in diameter, and but a single corpuscle of this minute size was measured.

The number of corpuscles on each negative ranges from 50 to 140, except on a single negative, which presents only 26 corpuscles. This happens to be a group of large corpuscles the mean diameter of which is 343 millionths of an inch, being the largest average diameter obtained on any negative. The smallest average was 309 millionths of an inch, being the mean of 90 corpuscles.

The measurements of dog's blood are from thirteen negatives, taken from five drops of blood, each from a single individual. The largest corpuscle measured was 378 millionths of an inch in diameter; the smallest, 237 millionths of an inch. The negatives contain from 80 to 175 corpuscles each, the total number of corpuscles measured being 1571. The largest average size for any negative was 340 millionths of an inch, being the mean of 100 corpuscles. The smallest was 296 millionths of an inch, being the mean of 111 corpuscles. It will be observed that on seven of the negatives of dog's blood the corpuscles have an average diameter smaller than the corpuscles on any of the negatives of human blood, while on the other six the average size of the corpuscles proves to be larger than the smallest average for human blood, and the largest average on any one negative of dog's blood exceeds that for any negative of human blood except the very largest. I call attention also to the very diverse averages obtained with both the human and dog's blood from different parts of the very same drop.

The measurements of guinea-pig's blood are from four negatives only, made from different parts of a single drop of blood. The total number of corpuscles measured was 401. One of the negatives gives an average one-millionth of an inch smaller than the smallest average for human blood; all the others give averages larger than the smallest for human blood. The variations in size are not so great as in the negatives either of dog's blood or of human, but, as they are taken from a single drop, it can hardly be assumed that this is a characteristic feature of the blood of the guinea-pig. I think no one could have told from the examination of this drop of blood whether it belonged to the guinea-pig, the dog, or man.

TABLE I.

Measurements of Human Red Corpuscles from Eight Individuals.

	No. of Corpuscles measured.	Diameters of Human Blood.			
		Dec. of an Eng. Inch.			Dec. of a Millimetre.
		Maximum.	Minimum.	Mean.	Mean.
1. Drop H, Neg. 849	90	.000363	.000255	.000309	.007848
2. Drop G, " 846	55	.000353	.000245	.000311	.007899
3. Drop E, " 835	70	.000339	.000261	.000312	.007925
4. Drop D, " 828	140	.000346	.000255	.000314	.007975
5. Drop H, " 848	50	.000343	.000274	.000315	.008001
6. Drop C, " 824	50	.000337	.000273	.000316	.008026
7. Drop G, " 840	81	.000372	.000265	.000316	.008026
8. Drop G, " 841	104	.000363	.000255	.000317	.008052
9. Drop H, " 847	80	.000363	.000255	.000319	.008102
10. Drop D, " 827	90	.000364	.000218	.000320	.008128
11. Drop A, " 820	75	.000359	.000230	.000326	.008280
12. Drop B, " 822	105	.000368	.000258	.000326	.008280
13. Drop I, " 854	80	.000353	.000294	.000326	.008280
14. Drop C, " 823	75	.000360	.000261	.000326	.008280
15. Drop C, " 825	75	.000382	.000282	.000327	.008306
16. Drop B, " 821	105	.000368	.000285	.000327	.008306
17. Drop A, " 818	80	.000359	.000278	.000328	.008331
18. Drop I, " 855	70	.000382	.000284	.000331	.008407
19. Drop D, " 826	65	.000371	.000285	.000334	.008483
20. Drop F, " 837	60	.000396	.000216	.000335	.008509
21. Drop A, " 819	110	.000396	.000276	.000337	.008560
22. Drop E, " 836	26	.000378	.000288	.000343	.008712

TABLE II.

Measurements of Red Corpuscles of the Dog from Five Individuals.

	No. of Corpuscles measured.	Diameters of Dog's Blood.			
		Dec. of an Eng. Inch.			Dec. of a millimetre.
		Maximum.	Minimum.	Mean.	Mean.
1. Drop A, Neg. 815	111	.000352	.000257	.000296	.007518
2. Drop B, " 839	107	.000346	.000237	.000296	.007518
3. Drop E, " 859	120	.000363	.000245	.000298	.007569
4. Drop B, " 829	175	.000346	.000253	.000298	.007569
5. Drop E, " 860	120	.000353	.000255	.000301	.007645
6. Drop A, " 816	120	.000342	.000247	.000305	.007747
7. Drop C, " 831	152	.000355	.000246	.000308	.007823
8. Drop C, " 833	140	.000359	.000258	.000310	.007874
9. Drop D, " 857	100	.000353	.000265	.000310	.007874
10. Drop D, " 858	111	.000353	.000255	.000310	.007874
11. Drop A, " 817	80	.000361	.000238	.000315	.008001
12. Drop C, " 832	135	.000370	.000270	.000317	.008052
13. Drop C, " 834	100	.000378	.000270	.000340	.008636

TABLE III.

Measurements of Red Corpuscles of the Guinea-pig from One Individual.

	No. of Corpuscles measured.	Diam. of Guinea-pig's Blood.			
		Dec. of Eng. Inch.			Dec. of a Millimetre.
		Maximum.	Minimum.	Mean.	Mean.
1. Drop A, Neg. 852	111	.000363	.000265	.000308	.007823
2. Drop A, " 850	100	.000372	.000265	.000310	.007874
3. Drop A, " 851	90	.000353	.000253	.000313	.007950
4. Drop A, " 853	100	.000382	.000265	.000314	.007975

I propose to make a number of other negatives of the blood of the dog and the guinea-pig at an early day, with a view to additional measurements; but those now published are, I must think, quite sufficient to demonstrate the reckless temerity of those who would attempt to discriminate human blood, even on nicely-dried slides prepared with every possible care to preserve the shape of the corpuscles.